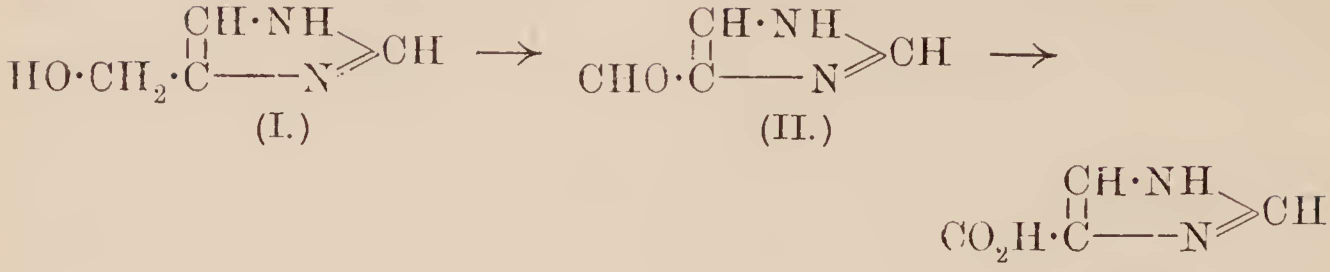
170

XXII.—*Derivatives of Glyoxaline-4(or 5)-formaldehyde and Glyoxaline-4(or 5)-carboxylic Acid. A New Synthesis of Histidine.*

By Frank Lee Pyman.

Some of the simpler substituted glyoxalines are little known. The simplest aldehyde in this field, glyoxaline-4(or 5)-formaldehyde (II), was obtained by the author (T., 1912, **101**, 542) in a yield of 15 per cent. of the theoretical by the oxidation of 4(or 5)-hydroxymethylglyoxaline (I) by means of chromic acid. As the analogue of benzaldehyde, the starting material for many syntheses in the aromatic series, this substance is of some importance. Its preparation and some of its reactions have therefore been studied.

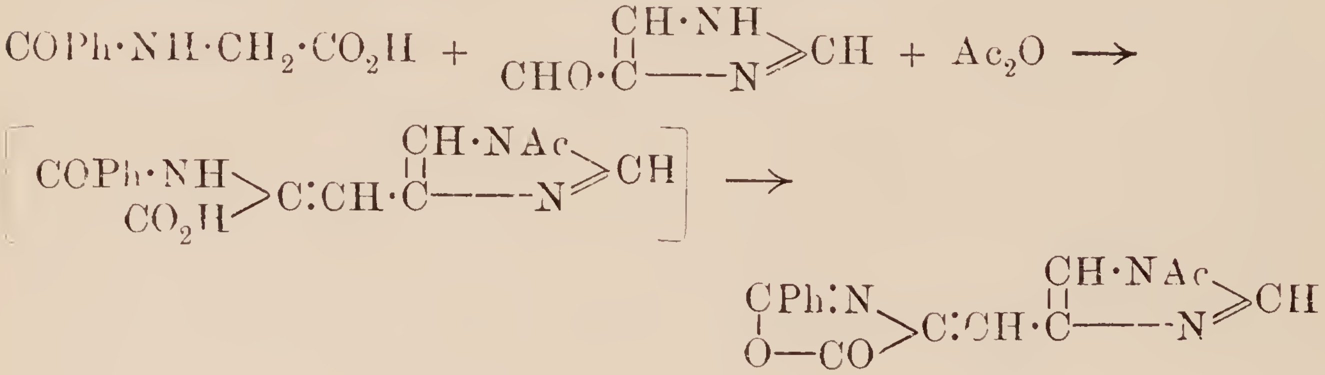
Glyoxalineformaldehyde may be obtained in a yield of more than 50 per cent. of the theoretical by the oxidation of hydroxymethylglyoxaline with little more than the calculated quantity of nitric acid, and its stability towards oxidising agents is such that it is obtained in a small yield, together with large quantities of glyoxaline-4(or 5)-carboxylic acid (III), when the alcohol is oxidised with a very large excess of nitric acid over the quantity required for its conversion into the acid.



$$\mathrm{HO\cdot CH_2\cdot \overset{CH\cdot NH}{\overset{\|}{C}}\!-\!-\!N}\!>\!\mathrm{CH} \rightarrow \mathrm{CHO\cdot \overset{CH\cdot NH}{\overset{\|}{C}}\!-\!-\!N}\!>\!\mathrm{CH} \rightarrow \mathrm{CO_2H\cdot \overset{CH\cdot NH}{\overset{\|}{C}}\!-\!-\!N}\!>\!\mathrm{CH}$$

(I.) (II.) (III.)

Glyoxalineformaldehyde yields a *phenylhydrazone*, and a *cyanohydrin* which will be discussed later. It does not undergo Perkin's reaction for the synthesis of the acrylic acid in a satisfactory manner, decomposition taking place. On the other hand, it can be condensed very easily with hippuric acid by means of acetic anhydride and sodium acetate, yielding 2-*phenyl*-4-[1-*acetylglyoxaline*-4(or 5)-*methylidene*]-*oxazolone* (IV).



$$\mathrm{COPh\cdot NH\cdot CH_2\cdot CO_2H} + \mathrm{CHO\cdot \overset{CH\cdot NH}{\overset{\|}{C}}\!-\!-\!N}\!>\!\mathrm{CH} + \mathrm{Ac_2O} \rightarrow$$

$$\left[\begin{array}{l}\mathrm{COPh\cdot NH}\\ \mathrm{CO_2H}\end{array}\!\!>\!\mathrm{C{:}CH\cdot \overset{CH\cdot NAc}{\overset{\|}{C}}\!-\!-\!-\!N}\!>\!\mathrm{CH}\right] \rightarrow$$

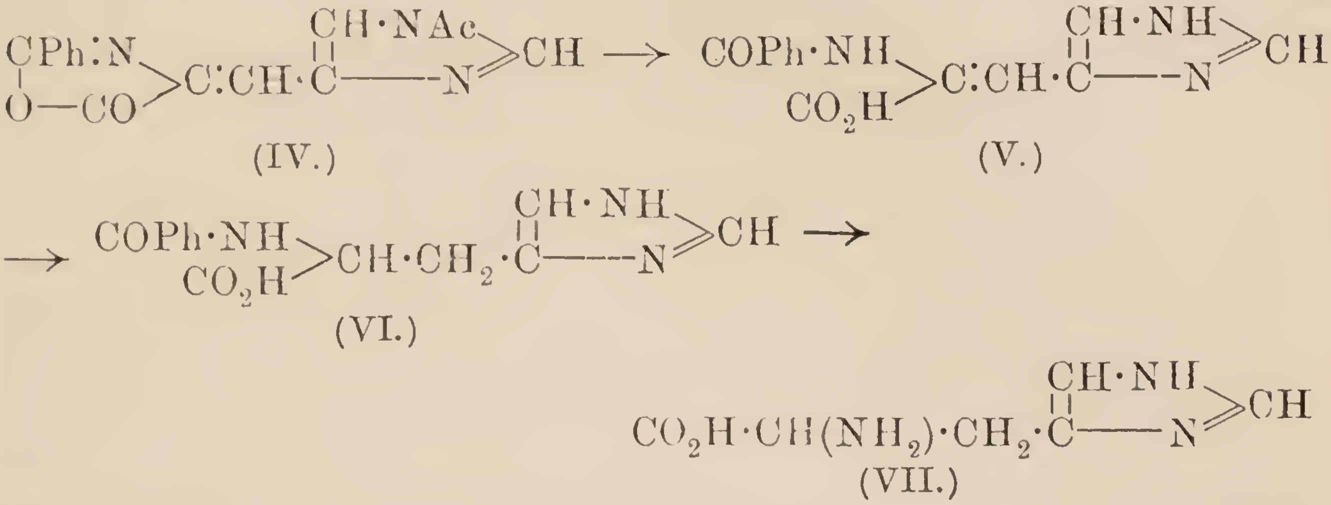
$$\begin{array}{l}\mathrm{CPh{:}N}\\ |\\ \mathrm{O{-}CO}\end{array}\!\!>\!\mathrm{C{:}CH\cdot \overset{CH\cdot NAc}{\overset{\|}{C}}\!-\!-\!-\!N}\!>\!\mathrm{CH}$$

(IV.)

This condensation has made it possible to effect a synthesis of histidine on the lines of the synthesis of phenylalanine carried out by Plöchl (*Ber.*, 1884, **17**, 1616) and Erlenmeyer, jun. (*Annalen*, 1893, **275**, 1), and that of tyrosine due to Erlenmeyer, jun., and Halsey (*Annalen*, 1899, **307**, 138).

The oxazolone (IV) is obtained in a yield of 68 to 76 per cent. of the theoretical. The acetyl group which it contains doubtless replaces the imino-hydrogen atom of the glyoxaline ring, for it is easily removed on hydrolysis, and there is, indeed, no other possible point of attachment. The formation of isomeric 1:4- and 1:5-acetyl derivatives would be expected on acetylation of a 4(or 5)-substituted glyoxaline, analogous to the formation of 1:4- and 1:5-dimethylglyoxaline on the methylation of 4(or 5)-methylglyoxaline (T., 1910, **97**, 1814), but the above described compound is homogeneous. In this connexion it may be noted that Gerngross (*Ber.*, 1913, **46**, 1908) has prepared the *N*-benzoyl derivatives of several 4(or 5)-substituted glyoxalines, and does not record the formation of isomerides in any case.

When the oxazolone is boiled with very dilute aqueous sodium carbonate, the acetyl group is removed and the oxazolone ring is opened. On then mixing the solution with the calculated quantity of an acid, *α-benzoylamino-β-glyoxaline-*4(or 5)-*acrylic acid* (V) is precipitated.



$$\begin{matrix}CPh{:}N\\ |\\ O{-}CO\end{matrix}\!>\!C{:}CH\cdot\overset{CH\cdot NAc}{\overset{\|}{C}}{-\!-\!-}N\!\geqslant\!CH \rightarrow \begin{matrix}COPh\cdot NH\\ CO_2H\end{matrix}\!>\!C{:}CH\cdot\overset{CH\cdot NH}{\overset{\|}{C}}{-\!-\!-}N\!\geqslant\!CH$$

(IV.) (V.)

$$\rightarrow \begin{matrix}COPh\cdot NH\\ CO_2H\end{matrix}\!>\!CH\cdot CH_2\cdot\overset{CH\cdot NH}{\overset{\|}{C}}{-\!-\!-}N\!\geqslant\!CH \rightarrow$$

(VI.)

$$CO_2H\cdot CH(NH_2)\cdot CH_2\cdot\overset{CH\cdot NH}{\overset{\|}{C}}{-\!-\!-}N\!\geqslant\!CH$$

(VII.)

The reduction of this acid to the saturated acid, *benzoyl*-r-*histidine* (VI), and hydrolysis of the latter to *r*-histidine (VII), were carried out according to E. Fischer's modifications (*Ber.*, 1899, **32**, 3638) of Erlenmeyer, jun.'s, methods for the treatment of the corresponding acids in the synthesis of tyrosine. Benzoyl-*r*-histidine has not been prepared previously. It crystallises from water with one molecular proportion of the solvent, and melts and decomposes at 248° (corr.) In both these respects it resembles benzoylhistidine, which also crystallises with $1H_2O$ according to Fränkel (*Beitr. chem. Physiol. Path.*, 1906, **8**, 156), who gives the melting point as 230°; Pauly, however (*Ber.*, 1910, **43**, 2254), states that it melts at 249° (corr.). The identity of *r*-histidine prepared by the above method was proved by comparison with a specimen previously synthesised by another method (T., 1911, **99**, 1386).

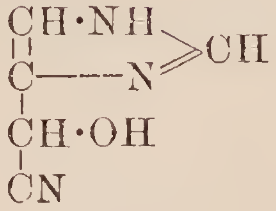
It has been stated above that glyoxalineformaldehyde yields a cyanohydrin (VIII). This compound is very unstable and readily loses hydrogen cyanide, regenerating the starting material. In a cold solution, however, it can be reduced by means of sodium amalgam and hydrochloric acid in nearly neutral solution (compare Meister, Lucius, & Brüning, D.R.-P. 193634 *) to give *β-hydroxy-*

---

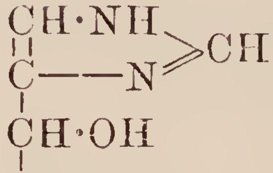
* Mannich and Thiele (*Arch. Pharm.*, 1915, **253**, 181) have recently described a method for the preparation of β-hydroxy-β-arylethylamines by the reduction of phenyl aminomethyl ketones with hydrogen in the presence of palladinised charcoal.

In discussing previous methods, they dismiss the method of D.R.-P. 193634 with the remark that it does not seem to be of practical importance, and they also overlook the fact that this patent describes the preparation of phenylethanolamine (β-hydroxy-β-phenylethylamine), which they claim to have prepared for the first time. As a matter of fact, this substance is described in the patent and can be prepared easily and quickly from benzaldehyde, as there stated, in a yield of more than 30 per cent. of the theoretical, whereas Mannich and Thiele's method involves the previous preparation of ω-aminoacetophenone (Mannich and Hahn, *Ber.*, 1911, **44**, 1542), for which ω-bromoacetophenone is required.

β-glyoxaline-4(or 5)-*ethylamine* (IX) in a yield amounting to 32 per cent. of the theoretical, calculated on the glyoxalineformaldehyde.



(VIII.)



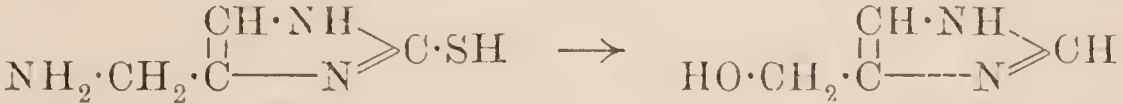
(IX.)

The new base was prepared in order to compare its physiological action with that of 4(or 5)-β-aminoethylglyoxaline, a base which occurs in extracts of ergot (Barger and Dale, T., 1910, **97**, 2592), and is of great physiological activity (Dale and Laidlaw, *J. Physiol.*, 1910, **41**, 318). Constitutionally, it differs from the latter only in containing a hydroxyl group, so that in the place of the ethylamine group it contains an ethanolamine group similar to that contained in adrenaline, the active principle of the suprarenal glands. The comparison of the ethylamine and ethanolamine derivatives of glyoxaline was interesting, because Barger and Dale (*J. Physiol.*, 1910, **41**, 19) have compared previously the ethylamine and ethanolamine derivatives of phenols. They showed that β-hydroxy-β-*p*-hydroxyphenylethylamine was inferior in its sympathomimetic action to β-*p*-hydroxyphenylethylamine, having only one-fifth of the power, whilst β-hydroxy-β-3:4-dihydroxyphenylethylamine is fifty times as powerful as β-3:4-dihydroxyphenylethylamine. In the present case, experiments kindly carried out by Dr. H. H. Dale, F.R.S., have shown that the introduction of the hydroxyl group has diminished the physiological activity, β-hydroxy-β-glyoxaline-4(or 5)-ethylamine being less active than 4(or 5)-β-aminoethylglyoxaline.

In the course of this work a considerable amount of glyoxaline-4(or 5)-carboxylic acid (III) was accumulated, and the opportunity was taken of studying its properties. This acid was first described by Knoop (*Beitr. chem. Physiol. Path.*, 1907, **10**, 111), who obtained it by the oxidation of glyoxaline-4(or 5)-glyoxylic acid, an oxidation product of histidine. Knoop stated that he had also prepared it synthetically from glyoxaline-4:5-dicarboxylic acid, which can be prepared from tartaric acid, and that the synthesis was to be described in another place. No further account of the work seems to have appeared. Windaus and Ullrich (*Zeitsch. physiol. Chem.*, 1914, **90**, 366) subsequently isolated the acid from the products of reaction of dextrose and ammoniacal copper hydroxide. Both authors agree that the acid melts and effervesces at about 286°, losing carbon dioxide and yielding glyoxaline.

Several salts of glyoxalinecarboxylic acid with acids have now been prepared, and also the *methyl* and *ethyl esters.* The esters behave in an abnormal manner towards sodium diazobenzene-*p*-sulphonate. Glyoxalines containing a free imino-hydrogen atom couple with this reagent in aqueous sodium carbonate, forming a carmine-coloured solution; glyoxalinecarboxylic acid itself behaves normally, but the esters fail to give a red colour. Gerngross (*Ber.*, 1912, **45**, 513) has previously noticed the same phenomenon with 5(or 4)-methylglyoxaline-4(or 5)-carboxylic acid and its esters; here also the acid couples, but the esters do not. Attempts to prepare the acid chloride of glyoxalinecarboxylic acid hydrochloride have so far proved quite unsuccessful, for the acid fails to react with thionyl chloride or with phosphorus pentachloride, either alone or when mixed with other acid chlorides even at high temperatures. In this connexion it is of interest to note that H. Meyer (*Monatsh.*, 1901, **22**, 415) found that whilst *o*- and *m*-hydroxybenzoic acids were readily converted into the acid chlorides by means of thionyl chloride, the para-isomeride remained unaltered.

In the course of purifying a quantity of hydroxymethylglyoxaline (XI), prepared by the action of nitric acid on 2-thiol-4(or 5)-aminomethylglyoxaline (X) (T., 1911, **99**, 669), a by-product * having



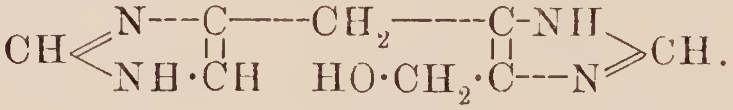
$$NH_2\cdot CH_2\cdot\begin{array}{l}CH\cdot NH\\ \|\\ C\text{——}N\end{array}\!\!>C\cdot SH \longrightarrow HO\cdot CH_2\cdot\begin{array}{l}CH\cdot NH\\ \|\\ C\text{——}N\end{array}\!\!>CH$$

(X). (XI.)

the empirical formula $C_8H_{10}ON_4$ was isolated. This compound can be coupled with sodium diazobenzene-*p*-sulphonate, giving a red dye, a characteristic of glyoxaline derivatives. It is a diacidic base forming well-crystallised neutral salts. Taking into account its mode of formation and properties, there can be little doubt that it contains two glyoxalinemethyl groups ($C_3H_3N_2\cdot CH_2\cdot$), which account for all the elements it contains except the one oxygen atom. It thus appeared that the substance was either glyoxalinemethyl ether, $(C_3H_3N_2\cdot CH_2)_2O$, or glyoxalinemethylglyoxalinemethyl alcohol, $C_3H_3N_2\cdot CH_2\cdot C_3H_2N_2\cdot CH_2\cdot OH$. In order to determine which formula was correct, the substance was heated under pressure with acids, when it was found to be unchanged. It did not therefore appear to be an ether. It was consequently presumed to have the alternative alcohol formula, and this view was shown to be correct in the following manner. The substance was heated with benzoyl chloride on the water-bath, when a *monobenzoyl* derivative, $C_{15}H_{14}O_2N_4$, was obtained. The same method had been employed previously for the benzoylation of hydroxymethylglyoxaline, $C_3H_3N_2\cdot CH_2\cdot OH$ (T., 1912, **101**, 541), when glyoxalinemethyl benzoate, $C_3H_3N_2\cdot CH_2\cdot O\cdot COPh$, was obtained. The method did not, therefore, lead to the formation of an *N*-benzoylglyoxaline derivative. Such compounds are only obtained under special conditions, and are unstable in moist air (Gerngross, *Ber.*, 1913, **46**, 1908). The benzoyl derivative, $C_{15}H_{14}O_2N_2$, is stable, and can be recrystallised from water without decomposition. These facts show that the benzoyl derivative is not a *N*-benzoyl, but an *O*-benzoylglyoxaline derivative, and indicate that it is glyoxalinemethylglyoxalinemethyl benzoate. The formation of glyoxalinemethylglyoxalinemethyl alcohol is due to the displacement of one of the hydrogen atoms of hydroxymethylglyoxaline by the glyoxalinemethyl group. Now, the condensation of 4(or 5)-methylglyoxaline with formaldehyde leads to 4(or 5)-methyl-5(or 4)-hydroxymethylglyoxaline, the substituent taking up the 5(or 4)-position (Windaus, *Ber.*, 1909, **42**, 758; Ewins, T., 1911, **99**, 2052), and it seems probable that in the present case also the substituent group occupies the corresponding position. The substance is thus in all probability 4(or 5)-[*glyoxaline*-4(or 5)-*methyl*]-*glyoxaline*-5(or 4)-*methyl alcohol* (XII):

---

* The occurrence of this base as a by-product in the preparation of hydroxymethylglyoxaline was discovered by Mr. W. H. Taylor, B.Sc., who kindly drew the author's attention to it.



$$CH\Bigg\langle\begin{array}{l}N{-}{-}{-}\overset{}{C}{-}{-}{-}{-}CH_2{-}{-}{-}{-}C{-}NH\\ \quad\ \ \ \|\qquad\qquad\qquad\quad\ \ \|\\ NH\cdot CH\quad HO\cdot CH_2\cdot C{-}{-}{-}N\end{array}\Bigg\rangle CH.$$

(XII.)

EXPERIMENTAL.

Oxidation of 4(or 5)-*Hydroxymethylglyoxaline.*

Hydroxymethylglyoxaline is readily oxidised by means of concentrated nitric acid, giving a mixture of glyoxalineformaldehyde and glyoxalinecarboxylic acid. The relative amounts of each of these substances obtained depend on the proportion of nitric acid employed, and methods have been worked out for preparing both compounds in good yield.

(*a*) *Preparation of Glyoxalineformaldehyde.*—Twenty-six grams of hydroxymethylglyoxaline and 38 c.c. of nitric acid (D 1·42) were digested in a covered dish on the water-bath until the evolution of brown fumes was almost complete. The cover was then removed and the liquid evaporated, when a crystalline mass remained. This was dissolved in a warm concentrated solution of sodium carbonate and kept, when 11·3 grams of glyoxalineformaldehyde (m. p. 170°) crystallised. After removing the crystals, the filtrate was acidified faintly to methyl-orange with hydrochloric acid and kept, when 5·1 grams of glyoxalinecarboxylic acid separated. This was collected and the filtrate evaporated nearly to dryness. The residue was mixed with alcohol, filtered from inorganic salts, and evaporated to remove the alcohol. This residue was dissolved in warm concentrated aqueous sodium carbonate and kept, when 3·1 grams of glyoxalineformaldehyde (m. p. 170°) crystallised. The yields of the aldehyde and the acid are 56 and 17 per cent. of the theoretical respectively.

(*b*) *Preparation of Glyoxalinecarboxylic Acid.*—Seventeen grams of hydroxymethylglyoxaline were dissolved in 100 c.c. of concentrated nitric acid (three times the quantity calculated for its conversion into the nitrate of glyoxalinecarboxylic acid) and warmed on the water-bath in a covered dish until the evolution of brown fumes was almost complete. The liquor was then evaporated to dryness on the water-bath and the crystalline residue dissolved in water. A sufficient quantity (about 45 c.c.) of a cold saturated aqueous solution of sodium carbonate was added until the liquor was only slightly acid to methyl-orange. This caused the separation of the bulk of the glyoxalinecarboxylic acid (10·6 grams) as a colourless, crystalline powder which melted and decomposed at 281°. After the removal of this crop, the filtrate and washings were evaporated to a small volume and treated with sodium carbonate until neutral to methyl-orange, a further crop of 3·0 grams of glyoxalinecarboxylic acid (m. p. 275°) being thus obtained. The mother liquor was added to a solution of 12 grams of picric acid in 300 c.c. of boiling water, and after keeping overnight 6·4 grams of glyoxalineformaldehyde picrate (m. p. 191°) separated. The mother liquor was concentrated to half volume and kept, when glyoxalinecarboxylic acid picrate crystallised out mixed with a little picric acid and sodium picrate. It was purified by recrystallisation from water, when 5·0 grams of the pure salt were obtained.

The total yields represent 15·3 grams of glyoxalinecarboxylic acid and 1·9 grams of glyoxalineformaldehyde, that is, 82 and 11 per cent. of the theoretical respectively.

(*c*) An oxidation was also carried out with a quantity of nitric acid only slightly greater (using 3·5 instead of 3·0 molecular proportions) than that calculated for the conversion of hydroxymethylglyoxaline into glyoxalinecarboxylic acid nitrate. In this case the yields of acid and aldehyde were 55 and 29 per cent. of the theoretical respectively.

Glyoxaline-4(or 5)-*formaldehyde*,* $C_3H_3N_2 \cdot CHO$.

This base and its acid oxalate and picrate were described briefly in a previous paper (T., 1912, **101**, 542). Glyoxalineformaldehyde is not easily oxidisable; it is stable in the air and does not reduce ammoniacal silver nitrate. Its aqueous solution gives with this reagent a white precipitate like that of other glyoxalines containing a free imino-group, but this precipitate remains unchanged when the ammoniacal silver nitrate in which it is suspended is boiled. Glyoxalineformaldehyde gives a red dye with sodium diazobenzene-*p*-sulphonate. An attempt to convert glyoxalineformaldehyde into glyoxalineacrylic acid by boiling with fused sodium acetate in acetic anhydride was unsuccessful, for decomposition took place, the liquor rapidly darkening and soon becoming black.

The *phenylhydrazone*, $C_3H_3N_2 \cdot CH{:}N \cdot NHPh$, separated with the evolution of heat as a white, crystalline powder when 0·5 gram of the base and 0·6 c.c. of phenylhydrazine were ground together. After the mixture had been gently warmed on the water-bath to complete the reaction it was diluted with ether and filtered, when 0·85 gram of the hydrazone was obtained, melting at 187—190°. After crystallisation from alcohol, it formed prisms melting at 199—200° (corr.). It is sparingly soluble in water, alcohol, or acetone:

Found, C=64·0; H=5·4; N=30·3.

$C_{10}H_{10}N_4$ (186·1) requires C=64·5; H=5·4; N=30·3 per cent.

2-*Phenyl*-4-[1-*acetylglyoxaline*-4(or 5)-*methylidene*]-*oxazolone*, $C_{15}H_{11}O_3N_3$.

2·5 Grams of glyoxalineformaldehyde, 4·5 grams of hippuric acid, and 2·5 grams of fused sodium acetate were ground together, mixed with 10 c.c. of acetic anhydride, and heated on the water-bath. The solids quickly dissolved, giving a clear, dark brown, fused mass, which soon began to deposit well-formed, prismatic, yellow needles. After heating for half an hour the mass was cooled, filtered off, and washed with alcohol. The crystals were then ground with cold water, washed with water and alcohol, and dried in the air. The yield amounted to from 5·0 to 5·6 grams, that is, 68 to 76 per cent. of the theoretical, and the product was pure, the melting point remaining unchanged after recrystallisation of the substance.

---

* By the oxidation of histidine, Fränkel (*Beitr. chem. Physiol. Path.*, 1907, **10**, 116) obtained a neutral by-product melting at 300° together with glyoxaline-4(or 5)-glyoxylic acid. He suggested that this was probably glyoxalineformaldehyde, but it is now clear that it could not have been so, since the pure substance melts at 173—174° (corr.).

The *oxazolone* crystallises from chloroform in bright yellow needles melting at 191° (corr.). It is insoluble in water, very sparingly soluble in cold alcohol, ether, acetone, or benzene, sparingly so in cold chloroform, but readily so in hot chloroform:

Found, C = 64·0, 63·5; H = 3·9, 3·9; N = 15·2, 15·0.

$C_{15}H_{11}O_3N_3$ (281·1) requires C = 64·0; H = 3·9; N = 14·9 per cent.

It is gradually dissolved by boiling with hot alcohol, but appears to decompose under these conditions. It does not readily dissolve in cold dilute acids or alkalis. When covered with 10 per cent. aqueous hydrochloric acid, it is converted into a pale yellow powder, and on diluting the mixture and filtering, a clear, colourless filtrate results. When this is basified with aqueous sodium carbonate, a bright yellow colour is developed at first, and then an amorphous, yellow precipitate is deposited. The oxazolone is decomposed by heating with dilute mineral acids, forming benzoic and acetic acids, and presumably also glyoxalinepyruvic acid and ammonia. It yields α-benzoylamino-β-glyoxaline-4(or 5)-acrylic acid in good yield when boiled with dilute aqueous sodium carbonate, and is decomposed with evolution of ammonia when boiled with aqueous sodium hydroxide.

α-Benzoylamino-β-glyoxaline-4(or 5)-*acrylic Acid*, $C_{13}H_{11}O_3N_3$.

Five grams of the oxazolone were boiled with a solution of 2·5 grams of anhydrous sodium carbonate in 125 c.c. of water until completely dissolved (about fifteen minutes). The solution was treated with animal charcoal, filtered, and mixed with 1·76 grams of glacial acetic acid. The liquor set to a mass of colourless crystals, which were collected, washed, and dried in the air. The yield was 4·3 grams of the hydrated acid, that is, 88 per cent. of the theoretical.

α-Benzoylamino-β-glyoxaline-4(or 5)-*acrylic acid* crystallises from water in fine, colourless, glistening needles, which, after drying at 100°, melt and decompose at 208° (corr.). It is sparingly soluble in hot water and very sparingly so in cold water or alcohol. It is readily soluble in cold dilute mineral acids or alkalis, but not in dilute acetic acid:

Found, air-dried substance lost 6·7 per cent. at 100°.

$C_{13}H_{11}O_3N_3,H_2O$ requires H_2O = 6·5 per cent.

Found, in substance dried at 100°, C = 60·4; H = 4·5; N = 16·4.

$C_{13}H_{11}O_3N_3$ (257·1) requires C = 60·7; H = 4·2; N = 16·3 per cent.

Aqueous solutions of this acid immediately reduce cold aqueous potassium permanganate, and solutions in aqueous sodium carbonate give a deep red coloration with aqueous sodium diazobenzene-*p*-sulphonate.

The *hydrochloride* crystallises from water in bundles of prismatic needles. It is anhydrous and melts at 229° (corr.), turning brown and then black. It is readily soluble in cold, very easily so in hot water:

Found, C = 53·0; H = 4·2; Cl = 11·9.

$C_{13}H_{11}O_3N_3,HCl$ (293·6) requires C = 53·1; H = 4·1; Cl = 12·1 per cent.

The *picrate* crystallises from water in rosettes of yellow prisms, which after drying at 100° melt and decompose at 212° (corr.). It is fairly readily soluble in hot water, sparingly so in cold.

Benzoyl-r-*histidine*, $C_{13}H_{13}O_3N_3$.

9·7 Grams of α-benzoylamino-β-glyoxaline-4(or 5)-acrylic acid suspended in 97 c.c. of water were shaken with 97 grams of 2 per cent. sodium amalgam, which was added gradually during three-quarters of an hour without cooling the solution. The clear, pale brown liquor was decanted from the mercury, mixed with 21 c.c. of 33 per cent. aqueous sodium hydroxide, and boiled until ammonia was no longer evolved, and subsequently for ten minutes. The solution was digested with animal charcoal, filtered, and neutralised to litmus with hydrochloric acid, when 3·65 grams of benzoyl-*r*-histidine (m. p. 244°) crystallised out. The mother liquor on concentration gave a second crop of 1·3 grams (m. p. 232°). The crude product was recrystallised from water, and gave 4·1 grams of pure benzoyl-*r*-histidine, that is, 42 per cent. of the theoretical yield.

Benzoyl-r-*histidine* crystallises from water in hard, glistening prisms containing one molecular proportion of water of crystallisation, which is lost at 115°, but not at 100°. It melts and decomposes at 248° (corr.) after turning yellow from about 235°. It is somewhat sparingly soluble in hot water, very sparingly so in cold water or alcohol. It is readily soluble in cold dilute mineral acids or alkalis, but not in dilute acetic acid:

Found, in air-dried substance, loss at 100° = nil; loss at 115° = 6·8.

$C_{13}H_{13}O_3N_3,H_2O$ requires H_2O = 6·5 per cent.

Found, in substance dried at 115°, C = 60·2; H = 5·1; N = 16·2.

$C_{13}H_{13}O_3N_3$ (259·1) requires C = 60·2; H = 5·1; N = 16·2 per cent.

Aqueous solutions of this acid do not readily reduce cold aqueous potassium permanganate. A solution of the acid in aqueous sodium carbonate gives a deep red coloration with sodium diazobenzene-*p*-sulphonate.

The *hydrochloride* crystallises from water in hard, glistening prisms, which melt and effervesce at 232° (corr.). It is anhydrous, and is readily soluble in cold, very easily so in hot water:

Found, C=52·7; H=4·9; Cl=11·9.

$C_{13}H_{13}O_3N_3,HCl$ (295·6) requires C=52·8; H=4·8; Cl=12·0 per cent.

The *picrate* crystallises from water in bunches of glistening, feathery, yellow needles which, after drying at 100°, melt and decompose at 226° (corr.). It is fairly readily soluble in hot water, sparingly so in cold.

Hydrolysis of Benzoyl-r-*histidine. Formation of* r-*Histidine.*

Two grams of benzoyl-*r*-histidine and 120 c.c. of 20 per cent. aqueous hydrochloric acid were boiled for four hours under a reflux condenser. The solution was cooled and extracted with ether to remove the benzoic acid formed, and then evaporated to dryness in a partial vacuum. The residue, dissolved in a little water and mixed with a solution of hydrogen chloride in alcohol, deposited crude, crystalline *r*-histidine dihydrochloride melting at 210°. In order to purify this substance it was dissolved in water, and the solution was neutralised with aqueous sodium carbonate, digested with animal charcoal, filtered, and mixed with a hot concentrated aqueous solution of 3·3 grams of picric acid. On cooling, 3·1 grams of *r*-histidine dipicrate separated, and a further quantity of 0·4 gram was obtained from the mother liquors. The total yield, 3·5 grams, of this substance represents 70 per cent. of that theoretically possible. *r*-Histidine dipicrate sintered from 100° and melted at 103° (corr.) after drying in the air. A specimen of the same substance previously synthesised by the action of ammonia on α-chloro-β-glyoxaline-4(or 5)-propionic acid, and a mixture of the two, behaved similarly. After drying at temperatures below 100° both substances melted indefinitely between 140° and 150°, and the conditions of drying necessary for the salt to melt at 190° (corr.) were not rediscovered (compare T., 1911, **99**, 1397). On analysis the salt was found to have the composition previously recorded. (Found, C=33·2; H=3·0; H_2O=5·6. $C_6H_9O_2N_3,2C_6H_3O_7N_3,2H_2O$ requires C=33·3; H=3·0; H_2O=5·5 per cent.)

For complete identification of the substance, the picrate was converted into the dihydrochloride, and this into the base in the usual manner. The properties of both compounds agreed with those previously recorded for *r*-histidine prepared by the action of ammonia on α-chloro-β-glyoxaline-4(or 5)-propionic acid. Thus, analysis of the dihydrochloride gave the figures C=31·6; H=4·9; Cl=30·9; $C_6H_9O_2N_3,2HCl$ requiring C=31·6; H=4·9; Cl=31·1 per cent. This salt melted and decomposed at 235° (corr.), whilst a specimen prepared by the previous method and a mixture of the two specimens melted and decomposed at 237° (corr.) in the same bath. The free base crystallised from water in quadrilateral plates which decomposed at 284° (corr.).

Glyoxaline-4(or 5)-*formaldehydecyanohydrin*, $C_3H_3N_2\cdot CH(OH)\cdot CN$.

One gram of glyoxalineformaldehyde was dissolved in 2·5 c.c. of a saturated aqueous solution of sodium hydrogen sulphite. The solution became warm, and on the addition of a solution of 1·0 gram of potassium cyanide in 1·3 c.c. of water more heat was developed. On stirring and cooling, the solution quickly deposited the cyanohydrin in colourless, sandy crystals, which were collected, washed with a few drops of water, and drained on porous porcelain. The crude product amounted to 1·05 grams, and melted at 105°. It was quickly recrystallised from water, and a small portion separated in rosettes of prismatic needles which melted and decomposed at 115° (corr.):

Found, in air-dried substance, C=49·1; H=4·3.

$C_5H_5ON_3$ (123·1) requires C=48·8; H=4·1 per cent.

This substance is readily soluble in warm water, but sparingly so in ether or chloroform. It is easily decomposed when heated either alone or with solvents, yielding glyoxalineformaldehyde.

β-*Hydroxy*-β-*glyoxaline*-4(or 5)-*ethylamine*, $C_3H_3N_2\cdot CH(OH)\cdot CH_2\cdot NH_2$.

Three grams of glyoxalineformaldehyde were converted into the cyanohydrin by the method given above, and the crude product was reduced by the process employed by Meister, Lucius, & Brüning (D.R.-P. 193634) for the reduction of protocatechualdehydecyanohydrin to β-3:4-trihydroxyphenylethylamine. The cyanohydrin was dissolved in 40 c.c. of 2·5 per cent. aqueous hydrochloric acid and stirred at −5° to −3°, whilst 90 grams of 4 per cent. sodium amalgam were added gradually and sufficient (about 120 c.c.) 5 per cent. aqueous hydrochloric acid was added simultaneously to keep the solution as nearly neutral as possible. The operation was complete in one hour. The aqueous solution was then filtered and mixed with 1 litre of cold saturated aqueous picric acid. 6·1 Grams of the required picrate separated almost immediately in a pure state, the melting point being unchanged after recrystallisation of the salt from water. This yield is 32 per cent. of the theoretical calculated on the glyoxalineformaldehyde.

β-Hydroxy-β-glyoxaline-4(or 5)-*ethylamine dipicrate* crystallises from water in orange-yellow hexagonal plates or in serrated spikes. It is fairly readily soluble in hot, sparingly so in cold water. It contains $1H_2O$, which is not lost at 120°, and on heating sinters slightly from about 165° and melts and decomposes at 225° (corr.):

Found, in salt dried at 100°, C = 34·1, 33·9, 33·8; H = 2·9, 3·0, 2·8.

$C_5H_9ON_3,2C_6H_3O_7N_3,H_2O$ (603·2) requires C = 33·8; H = 2·8 per cent.

The *dihydrochloride* was prepared by decomposing the picrate with hydrochloric acid, removing the picric acid with ether, evaporating the acid solution to low bulk, and adding absolute alcohol. The salt crystallised in colourless needles, which melted and decomposed at 216° (corr.). It is anhydrous and is readily soluble in water, giving an acid solution, but insoluble in absolute alcohol:

Found, C = 30·5, 30·6; H = 5·5, 5·6; N = 21·5; Cl = 35·3.

$C_5H_9ON_3,2HCl$ (200·0) requires C = 30·0; H = 5·5; N = 21·0; Cl = 35·5 per cent.

This salt gives a deep red colour with sodium diazobenzene-*p*-sulphonate in alkaline solution. It gives no precipitate in aqueous solution with platinic chloride, gold chloride, mercuric chloride, or Meyer's reagent.

The *platinichloride* was prepared by mixing equivalent quantities of concentrated aqueous solutions of the dihydrochloride and platinic chloride and adding alcohol, when it separated in microscopic orange prisms containing $3H_2O$. (Found, H_2O = 8·8. Calc., H_2O = 9·1 per cent.) It begins to darken at 225° and gradually blackens up to 244° (corr.), when decomposition of the solid mass occurs. It is readily soluble in cold water, but very sparingly so in alcohol:

Found, Pt = 36·3.

$C_5H_9ON_3,H_2PtCl_6$ requires Pt = 36·3 per cent.

Glyoxaline-4(or 5)-*carboxylic Acid*, $C_3H_3N_2{\cdot}CO_2H$.

This substance crystallises from water in bunches of slender needles, which attain some length when crystallisation is slow. When placed in a bath at 100° and heated slowly, it melts and effervesces at 275° (corr.); when placed in a bath at 270°, at 284° (corr.). The melted product resolidifies on cooling, and then melts at 88°, the melting point of glyoxaline. Knoop (*loc. cit.*), who previously observed the formation of glyoxaline in this way, gives the melting point of the acid as 286°. His statement that the acid is readily soluble in water requires correction, for it is only sparingly soluble in cold water, although readily so in hot. Thus 1·0 gram of the acid required about 20 c.c. of boiling water for complete solution, and, on cooling, 0·86 gram crystallised from the solution. The acid is therefore soluble to the extent of less than 1 per cent. in cold water.

The acid is anhydrous. (Found, C = 42·8; H = 3·7. Calc., C = 42·9; H = 3·6 per cent.) It forms salts both with acids and bases. The *sodium* salt crystallises well.

The *hydrochloride* crystallises from water in clusters of microscopic prisms, which are anhydrous. It effervesces at 262° (corr.) and the molten residue crystallises on cooling. This salt is readily soluble in cold, very readily so in hot water:

Found, C = 32·5; H = 3·4; Cl = 23·9.

$C_4H_4O_2N_2$,HCl (148·5) requires C = 32·3; H = 3·4; Cl = 23·9 per cent.

The *nitrate* crystallises from water in prisms, which are anhydrous. It effervesces at 200°, then resolidifies, and melts again at about 270°. It is readily soluble in cold, very readily so in hot water:

Found, C = 27·7; H = 3·0.

$C_4H_4O_2N_2$,HNO_3 (175·1) requires C = 27·4; H = 2·9 per cent.

The *picrate* crystallises from hot water in small plates containing $1\frac{1}{2}H_2O$ (Found, H_2O = 7·5. Calc., H_2O = 7·3 per cent.), which are lost at 100°. After drying at 100°, it begins to melt at 195°, and forms a clear liquid at 215° (corr.), then effervesces. It is readily soluble in hot, sparingly so in cold water:

Found in salt dried at 100°, C = 35·2; H = 2·2.

$C_4H_4O_2N_2$,$C_6H_3O_7N_3$ (341·1) requires C = 35·2; H = 2·1 per cent.

Glyoxalinecarboxylic acid dissolved in aqueous sodium carbonate couples with sodium diazobenzene-*p*-sulphonate, giving a carmine dye, but its methyl and ethyl esters fail to do so.

All attempts to form the acid chloride of glyoxalinecarboxylic acid by the action of thionyl chloride or phosphorus pentachloride, either alone or in the presence of phosphoryl chloride, acetyl chloride, etc., on the acid, its ethyl ester or their salts, were quite unsuccessful, the substances remaining unaltered.

Ethyl Glyoxaline-4(or 5)-*carboxylate*, $C_3H_3N_2{\cdot}CO_2Et$.

Five grams of glyoxalinecarboxylic acid hydrochloride were boiled with 25 c.c. of absolute alcohol for six hours under a reflux condenser, the liquor being saturated from time to time with dry hydrogen chloride. The salt gradually passed into solution, and at the end of this time the alcohol was removed by distillation. The residue was dissolved in a small quantity of water and precipitated with a saturated solution of potassium carbonate, when, on stirring, 3·6 grams of the ester were obtained as a sandy precipitate melting at 158°.

Ethyl glyoxaline-4(or 5)-*carboxylate* crystallises from alcohol in clusters of plates which melt at 162° (corr.). It is sparingly soluble in cold, more readily so in hot water, fairly readily soluble in cold alcohol and readily so in ether or chloroform. It is anhydrous:

Found, C = 51·2; H = 5·7.

$C_6H_8O_2N_2$ (140·1) requires C = 51·4; H = 5·7 per cent.

On hydrolysis by boiling with 10 per cent. aqueous hydrochloric acid, the acid is formed in quantitative yield.

Methyl Glyoxaline-4(or 5)-*carboxylate*, $C_3H_3N_2{\cdot}CO_2Me$.

This ester was prepared in a similar manner. It crystallises from methyl alcohol in clusters of plates which melt at 156° (corr.). Its solubilities are similar to those of the ethyl ester, except that it is more sparingly soluble in cold methyl and ethyl alcohols:

Found, C = 47·6; H = 4·8.

$C_5H_6O_2N_2$ (126·1) requires C = 47·6; H = 4·8 per cent.

4(or 5)-[*Glyoxaline*-4(or 5)-*methyl*]-*glyoxaline*-5(or 4)-*methyl Alcohol*,
$C_3H_3N_2{\cdot}CH_2{\cdot}C_3H_2N_2{\cdot}CH_2{\cdot}OH$.

This base was obtained as a by-product in the preparation of 4-(or 5)-hydroxymethylglyoxaline by the action of nitric acid on 2-thiol-4(or 5)-aminomethylglyoxaline (T., 1911, **99**, 673). After the crystallisation of a large quantity of hydroxymethylglyoxaline as hydrogen oxalate in well-formed crystals, the mother liquors deposited a powdery salt which melted at 80—120°. Forty grams of this salt were mixed with an excess of aqueous barium hydroxide, filtered, saturated with carbon dioxide, and again filtered. The liquor was acidified with hydrochloric acid, evaporated to a syrup, and stirred with absolute alcohol, when 7·2 grams of the hydrochloride of glyoxalinemethylglyoxalinemethyl alcohol were obtained in a pure, crystalline condition, whilst another 4·5 grams, equally pure, were isolated from the mother liquor.

The base was obtained by dissolving 5 grams of the hydrochloride in water, adding 4 grams of anhydrous potassium carbonate, evaporating to dryness, and extracting with absolute alcohol. The residue left on distilling the alcoholic extract crystallised on the addition of a little water, 4·3 grams of the air-dried base being obtained.

Glyoxalinemethylglyoxalinemethyl alcohol crystallises from water in colourless, silky needles which contain $2H_2O$. (Found, $H_2O=16·4$, 16·7. Calc., $H_2O=16·8$ per cent.) It loses $2H_2O$ at 100°, and then melts at 174° (corr.). It is readily soluble in hot, but sparingly so in cold water:

Found, C=54·3, 53·7; H=5·5, 5·6; N=31·5.

$C_8H_{10}ON_4$ (178·1) requires C=53·9; H=5·7; N=31·5 per cent.

The *hydrochloride* crystallises from water or dilute alcohol in prisms, which are anhydrous and melt at 178—179° (corr.). It is very readily soluble in water and very sparingly so in cold alcohol:

Found, C=38·7, 38·5; H=4·7, 4·8; Cl=28·3, 28·1.

$C_8H_{10}ON_4,2HCl$ (251·1) requires C=38·2; H=4·9; Cl=28·3 per cent.

The *hydrogen oxalate* crystallises from water with $1H_2O$. (Found, $H_2O=5·4$, 5·2. Calc., $H_2O=4·8$ per cent.) After drying, it melts and effervesces at 165° (corr.). It is sparingly soluble in cold, but readily so in hot water:

Found, in anhydrous salt, C=40·3; H=3·9.

$C_8H_{10}ON_4,2C_2H_2O_4$ (358·2) requires C=40·2; H=3·9 per cent.

The *picrate* separates in long, broad needles from hot water. It is anhydrous and melts at 197—198° (corr.). It is fairly readily soluble in boiling water, very sparingly so in cold water:

Found, C=38·1, 37·7; H=2·5, 2·7.

$C_8H_{10}ON_4,2C_6H_3O_7N_3$ (636·2) requires C=37·7; H=2·5 per cent.

Glyoxalinemethylglyoxalinemethyl alcohol was recovered unchanged after heating (1) for three hours at 170° with ten times its weight of concentrated hydrochloric acid, and (2) for three hours at 190° with twenty times its weight of 50 per cent. sulphuric acid.

Both the alcohol and its benzoate yield a carmine solution when mixed with sodium diazobenzene-*p*-sulphonate in the presence of sodium carbonate.

Glyoxalinemethylglyoxalinemethyl benzoate was prepared by the action of benzoyl chloride on the alcohol. The method of preparation was precisely similar to that of glyoxalinemethyl benzoate

(T., 1912, **101**, 541), except that the acid solution was precipitated by the addition of sodium carbonate, when the benzoate separated as an oil which soon crystallised. The crystals melted at 67—68° (corr.), and the same melting point was recorded after the substance had been crystallised from water, when it separated in needles containing water of crystallisation:

Found, $H_2O = 8{\cdot}7$.

$C_{15}H_{14}O_2N_4,1\frac{1}{2}H_2O$ requires $H_2O = 8{\cdot}7$ per cent.

After crystallisation from ethyl acetate, the substance is obtained anhydrous in the form of warts melting at 120—121° (corr.):

Found, $C = 63{\cdot}9$; $H = 5{\cdot}0$; $N = 20{\cdot}0$.

$C_{15}H_{14}O_2N_4$ (282·1) requires $C = 63{\cdot}8$; $H = 5{\cdot}0$; $N = 19{\cdot}9$ per cent.

The substance is sparingly soluble in cold, but fairly readily so in hot water; readily soluble in alcohol or chloroform, but sparingly so in ether. It dissolves readily in dilute hydrochloric acid.

THE WELLCOME CHEMICAL RESEARCH LABORATORIES,
LONDON, E.C.

[*Received, February* 14*th*, 1916.]

PRINTED IN GREAT BRITAIN BY R. CLAY AND SONS, LTD.,
BRUNSWICK STREET, STAMFORD STREET, S.E., AND BUNGAY, SUFFOLK.